Topical Review

Signal transduction by G-proteins, Rho-kinase and protein phosphatase to smooth muscle and non-muscle myosin II

Andrew P. Somlyo*† and Avril V. Somlyo*‡

Departments of **Molecular Physiology and Biological Physics*, †*Medicine* (*Cardiology*) *and* ‡*Pathology, University of Virginia Health System, PO Box 800736, Charlottesville, VA 22908-0736, USA*

(Received 20 October 1999; accepted after revision 10 November 1999)

We here review mechanisms that can regulate the activity of myosin II, in smooth muscle and non-muscle cells, by modulating the Ca^{2+} sensitivity of myosin regulatory light chain (RLC) phosphorylation. The major mechanism of Ca^{2+} sensitization of smooth muscle contraction and non-muscle cell motility is through inhibition of the smooth muscle myosin phosphatase (MLCP) that dephosphorylates the RLC in smooth muscle and non-muscle. The active, GTP-bound form of the small GTPase RhoA activates a serine/threonine kinase, Rho-kinase, that phosphorylates the regulatory subunit of MLCP and inhibits phosphatase activity. G-protein-coupled release of arachidonic acid may also contribute to inhibition of MLCP acting, at least in part, through the Rho/Rho-kinase pathway. Protein kinase C(s) activated by phorbol esters and diacylglycerol can also inhibit MLCP by phosphorylating and thereby activating CPI-17, an inhibitor of its catalytic subunit; this mechanism is independent of the Rho/Rho-kinase pathway and plays only a minor, transient role in the G-protein-coupled mechanism of Ca^{2+} sensitization. Ca^{2+} sensitization by the Rho/Rho-kinase pathway contributes to the tonic phase of agonist-induced contraction in smooth muscle, and abnormally increased activation of myosin II by this mechanism is thought to play a role in diseases such as high blood pressure and cancer cell metastasis.

Myosin II, the major molecular motor of muscle and most non-muscle cells, is regulated not only by fluctuations in cytoplasmic calcium ($[Ca^{2+}]_i$), but also by other important signalling mechanisms. Thus, whereas excitation–contraction coupling in vertebrate striated muscles is under membrane potential control and contraction is initiated by binding of Ca^{2+} to a thin (actin) filament-associated protein, troponin, contractility of smooth muscle is regulated not only by such electromechanical coupling and $[Ca^{2+}]_i$, but also by membrane potential-independent, pharmacomechanical coupling (Somlyo & Somlyo, 1968; reviewed in Somlyo & Somlyo, 1994; Somlyo *et al.* 1999*a*). Furthermore, both smooth muscle and non-muscle myosin II are regulated by phosphorylation/dephosphorylation of the myosin regulatory light chain (RLC) by, respectively, Ca^{2+}–calmodulin-regulated myosin light chain kinase (MLCK) and myosin phosphatase (MLCP, also known as SMPP-1M); actin activation of myosin II is the result of RLC phosphorylation on Ser 19 (Adelstein & Conti, 1975; reviewed in Hartshorne, 1987; Tan *et al.* 1992; Somlyo & Somlyo, 1994; Gallagher *et al.* 1997). Therefore, it was to be expected that the Ca^{2+}-independent mechanisms that regulate smooth muscle myosin II will also regulate non-muscle myosin II and non-muscle motility (Somlyo & Somlyo, 1994). The small GTPase, RhoA, and its upstream activators and downstream effectors play a major role in these processes and are the subject of this brief review.

Increased RLC (Ser 19) phosphorylation of smooth and non-muscle myosin II can be effected not only by increasing $[Ca^{2+}]_i$ and, thereby, the activity of MLCK, but also by inhibiting MLCP. This was the basis of the suggestion (Somlyo *et al.* 1989), verified experimentally (Kitazawa *et al.* 1991), that contractions induced at constant $[Ca^{2+}]_i$ by certain agonists and by GTPγS were due to inhibition of a myosin phosphatase. The inhibitory signal for 'Ca^{2+} sensitization' is communicated by RhoA to a Rho-kinase that phosphorylates the $M_{110\text{-}130}$ regulatory subunit and inhibits the catalytic activity of MLCP, resulting in increased RLC phosphorylation, contraction and cell motility (Fig. 1). Experimental evidence that led to these conclusions is summarized below.

Although G-protein-coupled regulation of a myosin phosphatase was recognized over a decade ago (Somlyo *et al.* 1989), the regulated phosphatase (MLCP) that dephosphorylates the RLC of intact myosin was identified only

recently. It consists of a 110–130 kDa regulatory ($M_{110\text{-}130}$), an ~37 kDa catalytic (PP-1C; variously described as β or δ) subunit, and a 20 kDa subunit of unknown function (Alessi *et al.* 1992; Shirazi *et al.* 1994; Shimizu *et al.* 1994; Haystead *et al.* 1995; reviewed in Hartshorne *et al.* 1998). It is also present in non-muscle (e.g. Nakai *et al.* 1997; Murányi *et al.* 1998; Suzuki *et al.* 1999; Essler *et al.* 1999; reviewed in Hartshorne *et al.* 1998), including human prostate cancer

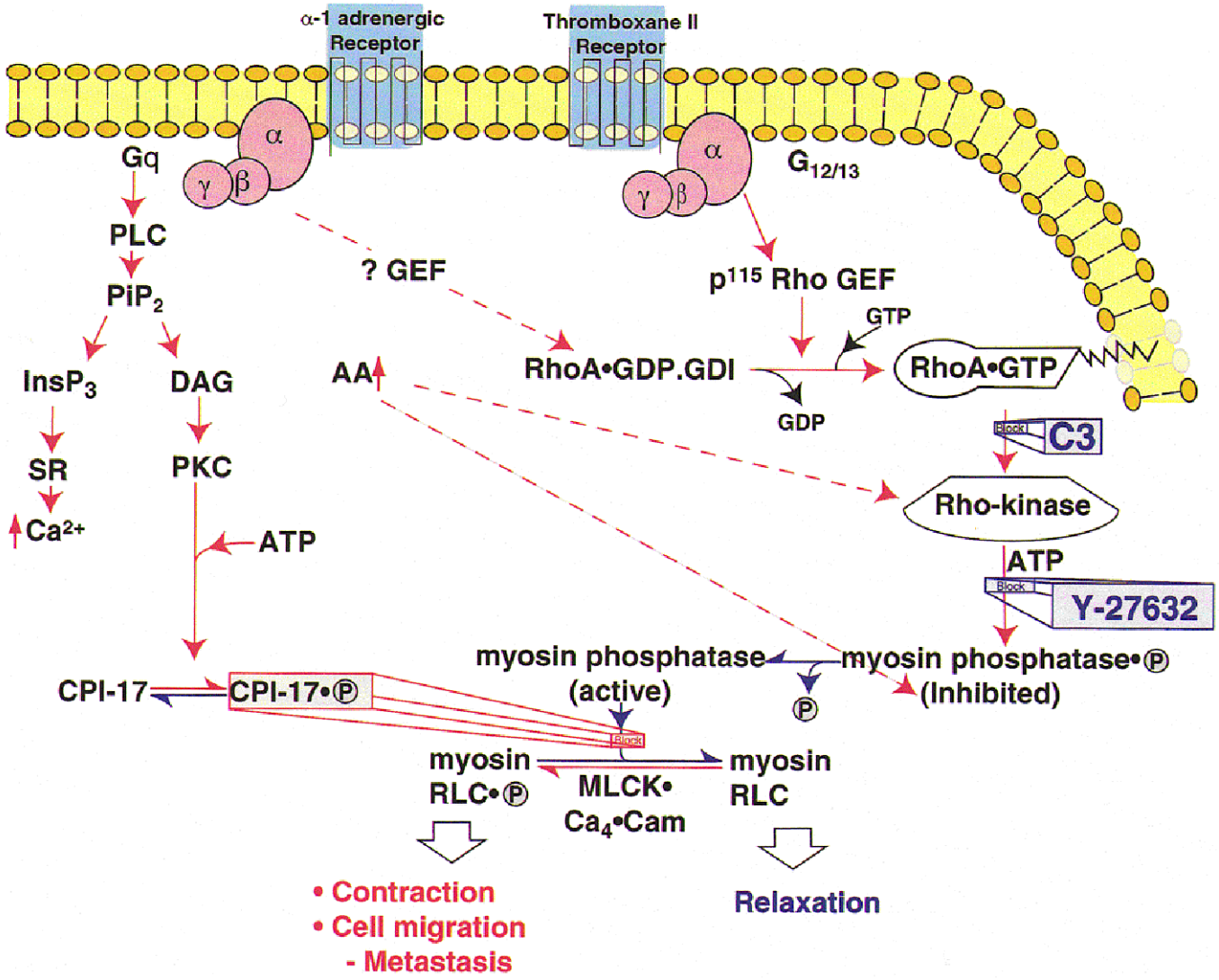

Figure 1. Regulation of myosin II in smooth and non-muscle cells

Pathways indicated in red activate myosin II, resulting in contraction, cell migration and cancer metastasis. Pathways that reduce myosin II activity are shown in blue. The major, Ca^{2+}-independent pathway that increases myosin II activity is through activation of Rho-kinase by RhoA·GTP, phosphorylation of the regulatory subunit of myosin phosphatase (MLCP) by Rho-kinase and some other kinases (see text), resulting in inhibition of MLCP activity and increased myosin RLC phosphorylation. Increases in arachidonic acid, due to a variety of stimuli, can also activate Rho-kinase and, at least *in vitro*, also inhibit MLCP activity by dissociating the regulatory ($M_{110\text{-}130}$) from the catalytic (PP-1C) subunit. The third pathway shown that enhances myosin II activity is through phosphorylation of CPI-17 by protein kinase C (PKC) leading to direct inhibition of PP-1C by the phosphorylated CPI-17 (CPI-17·P). Each of these mechanisms requires the presence of an active kinase that can phosphorylate Ser19 of RLC and increase myosin II activity by inhibiting MLCP even at constant $[Ca^{2+}]_i$ ('Ca^{2+} sensitization'). These mechanisms operate in parallel with and independently of the activation of MLCK by Ca^{2+} released from the sarcoplasmic reticulum/endoplasmic reticulum (SR/ER) by $InsP_3$ or by Ca^{2+} influx (not shown). Abbreviations: PLC, phospholipase C; PiP_2, phosphatidylinositol-bis-phosphate; $InsP_3$, inositol 1,4,5-trisphosphate; DAG, diacylglycerol; PKC, protein kinase C; GEF, guanine nucleotide exchange factor; MLCK, myosin light chain kinase; Cam, calmodulin; RLC, regulatory light chain; C3, *Clostridium botulinum*, exoenzyme that ADP-ribosylates Asn 41 of RhoA, inhibits RhoA activity and blocks its translocation to the membrane; Y-27632, selective inhibitor of Rho-kinase.

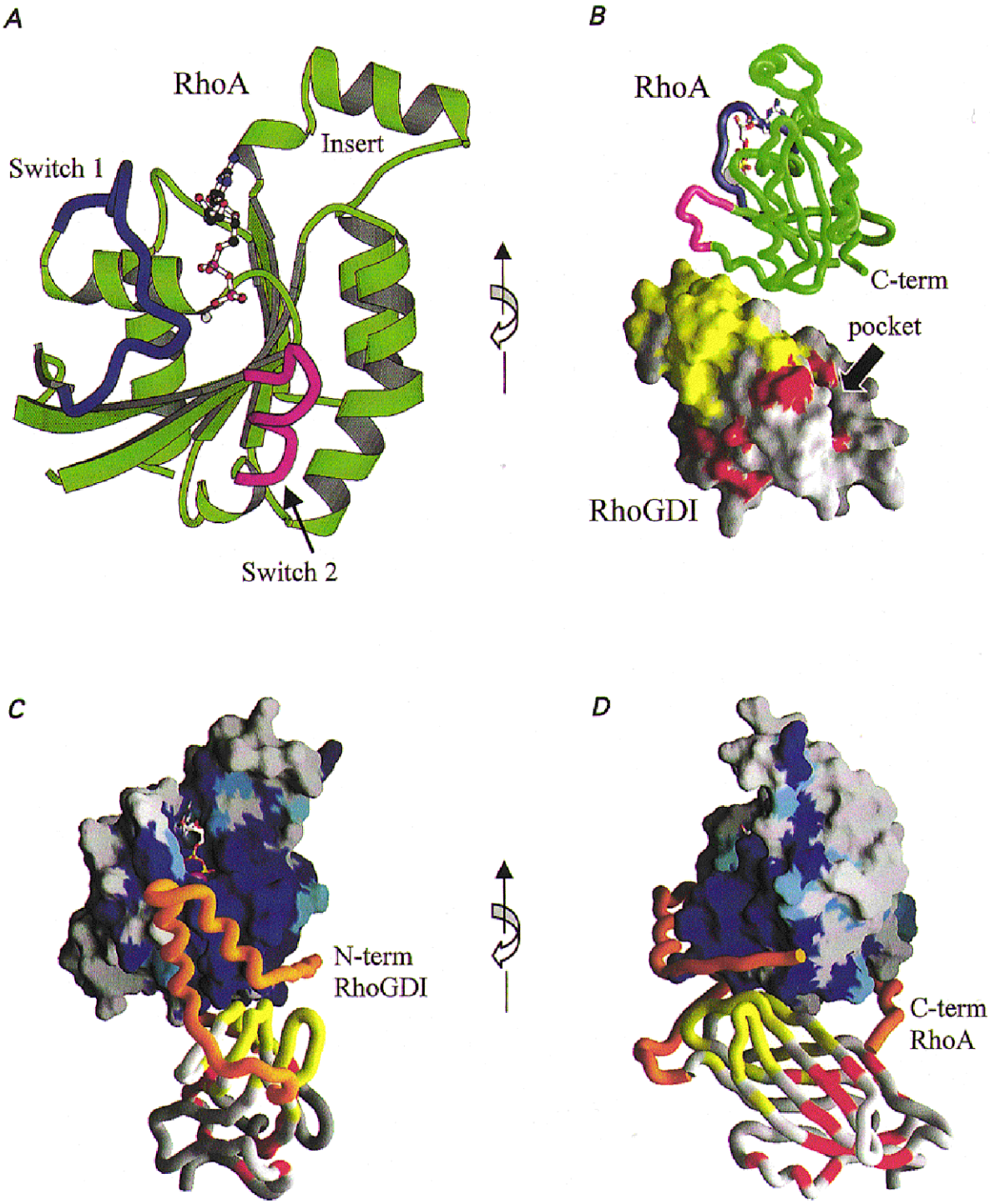

Figure 2. Structural features of the complex between RhoA and RhoGDI

A, ribbon drawing of the crystal structure of RhoA highlighting the switch 1 (blue) and switch 2 (magenta) regions (Wei *et al.* 1997). *B*, crystal structure of the RhoA–RhoGDI complex (Longenecker *et al.* 1999) reveals the mutual disposition of the individual structures of RhoA and RhoGDI. The length of the complex is approximately 77 Å (7·7 nm). The surface of RhoGDI is coloured according to resonance shifts observed in solution by NMR (Gosser *et al.* 1997). RhoGDI residues affected by addition of Cdc42 (unprenylated) are coloured yellow, and those affected by addition of a prenylated peptide are red. *C* and *D*, models (orange) of the N-terminal domain of RhoGDI (residues 24–68) and of the C-terminal extension of RhoA (residues 181–190) display additional structural features of the complex. The conservation of amino acids among the Rho-family (rho, rac, Cdc42) is depicted on the surface of RhoA as a gradation of blue, where dark blue represents 100 % conservation. The large conserved surface is consistent with the ability of RhoGDI to bind each of the above Rho-family GTPases. A ribbon drawing of RhoGDI is coloured as in *B*. The views in *B* and *D* are rotated about the vertical axis relative to *A* and *C*.

cells (Somlyo *et al.* 1999*b*). Selectivity of PP-1C for myosin is conferred by the $M_{110\text{-}130}$ subunit that potentiates dephosphorylation of myosin by PP-1C, *in vitro* (Alessi *et al.* 1992; Shirazi *et al.* 1994; Shimizu *et al.* 1994; reviewed in Hartshorne *et al.* 1998) and in permeabilized smooth muscle (Gailly *et al.* 1996). Phosphorylation of $M_{110\text{-}130}$ in its C-terminal half by Rho-kinase (Kimura *et al.* 1996) and some other kinase(s) on the same site (Feng *et al.* 2000) inhibits MLCP activity. Certain N-terminal fragments of $M_{110\text{-}130}$ can cause Ca^{2+} sensitization, probably through competitive inhibition of the endogenous protein (Zhou *et al.* 1999).

Agonists acting on receptors coupled to G_q induce both Ca^{2+} sensitization and activation of phospholipase C (PLC)-mediated hydrolysis of phosphatidylinositol-bis-phosphate to inositol-1,4,5-trisphosphate (InsP_3) and diacylglycerol to, respectively, cause Ca^{2+} release and activate protein kinase C (PKC); the concurrence of these effects implicated $G\alpha_q$ in both processes (Somlyo & Somlyo, 1994). However, Ca^{2+} release can be dissociated from Ca^{2+} sensitization (Kobayashi *et al.* 1991), and InsP_3 does not Ca^{2+} sensitize smooth muscle. These results exclude PLC products as major Ca^{2+}-sensitizing effectors of Gαq, consistent with the view that conventional and novel PKCs (for historical antecedents, see Andrea & Walsh, 1992; Somlyo *et al.* 1999*a*) play only a small and transient (Iizuka *et al.* 1999) or no role in G-protein-coupled Ca^{2+} sensitization (Jensen *et al.* 1996; Walker *et al.* 1998; Strassheim *et al.* 1999). Furthermore, some very potent Ca^{2+}-sensitizing agonists, such as U-46619, cause little or no detectable release of intracellular Ca^{2+} (Bradley & Morgan, 1987; Himpens & Somlyo, 1988; Himpens *et al.* 1990), as would be expected of receptors coupled to $G\alpha_{q/11}$. Subsequent studies of non-muscle cells revealed that RhoA can be activated by α subunits of other trimeric G-proteins, $G\alpha_{12,13}$, via linked guanine nucleotide exchange factors (GEFs; Hart *et al.* 1998; Kozasa *et al.* 1998; Gohla *et al.* 1999). The receptors activated by U-46619 are coupled to $G_{12,13}$ that, unlike $G\alpha_{q/11}$, do not activate PLC, suggesting that the disproportionately high Ca^{2+}-sensitizing (compared with Ca^{2+} releasing) effect of U-46619 (Himpens *et al.* 1990) is mediated by the $G\alpha_{12,13}$ family. A Gα-GAP (GTPase-activating protein), p^{115} Rho-GEF, interacts with $G\alpha_{13}$ and is probably the upstream 'convector' between this trimeric G-protein and RhoA (Kozasa *et al.* 1998; Hart *et al.* 1998). It now appears that several trimeric G-proteins, including $G\alpha_q$, $G\alpha_{12,13}$ and $G\alpha_{i\text{-}2}$, can activate RhoA, depending on the receptors and cell types involved (Katoh *et al.* 1998; Croxton *et al.* 1998; Klages *et al.* 1999; Hirshman & Emala, 1999).

RhoA, a monomeric G-protein, is, like most GTPases, active when it contains bound GTP and inactive when the bound nucleotide is GDP. RhoA·GTP does not directly inhibit MLCP, as indicated by its lack of effect on extensively permeabilized (with Triton X-100) smooth muscle (Gong *et al.* 1996), and Ca^{2+} sensitization requires its translocation to a relatively intact plasma membrane (Gong *et al.* 1997; Fujihara *et al.* 1997; Taggart *et al.* 1999). In resting smooth muscle (Gong *et al.* 1997) as in other cells (Bourmeyster *et al.* 1992; Abo *et al.* 1994; Bokoch *et al.* 1994), the cytosolic, inactive forms of RhoA and Rac are complexed with RhoGDI (guanine nucleotide dissociation inhibitor). The hydrophobic geranyl-geranylated tail of RhoA inserts into a hydrophobic cavity of GDI and binding is reinforced by protein–protein interactions between the N-terminus of GDI and a highly conserved (among Rho-family proteins) epitope of RhoA (Fig. 2; Gosser *et al.* 1997; Longenecker *et al.* 1999 and references therein). Cytosolic RhoA·RhoGDI is activated by Rho-GEFs (guanine nucleotide exchange factors) that stimulate nucleotide exchange on RhoA (GTP replaces GDP), followed by dissociation of RhoA·GTP from the complex and translocation to the plasma membrane, while GDI is retained in the cytosol (Gong *et al.* 1997; Fujihara *et al.* 1997; Read *et al.* 2000; reviewed in Cherfils & Chardin, 1999).

Bacterial exoenzymes that ADP-ribosylate Asn 41 (C3, EDIN) or monoglucosylate Thr 37 (*C. difficile* toxin B) of RhoA (reviewed in Sehr *et al.* 1998) block its biological activity; ADP-ribosylation also prevents its translocation to the plasma membrane (Gong *et al.* 1997; Fujihara *et al.* 1997; Croxton *et al.* 1998). These agents inhibit Ca^{2+} sensitization of RLC phosphorylation and force in smooth muscle (Noda *et al.* 1995; Itagaki *et al.* 1995; Gong *et al.* 1996; Fujihara *et al.* 1997; Otto *et al.* 1996) and stress fibre formation in non-muscle cells (reviewed in Hall, 1994).

The Ca^{2+}-sensitizing effector of RhoA·GTP is a serine/threonine-kinase (we refer to it generically as 'Rho-kinase') that contains a Rho-binding domain, has two identified isoforms and is activated by Rho·GTP (Leung *et al.* 1995; Ishizaki *et al.* 1996; Matsui *et al.* 1996). Both α and β isoforms are present in smooth muscle (Yoshii *et al.* 1999), with ROKα (ROCK-II) predominant in gizzard (Feng *et al.* 1999). The detailed mechanism of Rho-kinase activation by RhoA·GTP is not known. However, the requirement for RhoA to translocate to the plasma membrane to cause Ca^{2+} sensitization and the lack of Ca^{2+} sensitization by a recombinant, non-prenylated (*E. coli*-expressed) RhoA·GTP that does not bind to the membrane (Gong *et al.* 1996), suggest that activation of Rho-kinase by RhoA·GTP, in analogy with the Ras-activated Raf-kinase, occurs upon recruitment of both proteins to the plasma membrane (Leung *et al.* 1995). Activated Rho-kinase phosphorylates the regulatory subunit of MLCP and inhibits myosin phosphatase activity (Kimura *et al.* 1996). MLCP can also be phosphorylated on the same inhibitory site (Feng *et al.* 2000) and inhibited by other, yet unidentified kinases (Trinkle-Mulcahy *et al.* 1995; Ichikawa *et al.* 1996; reviewed in Hartshorne *et al.* 1998).

In addition to RhoA·GTP, arachidonic acid, a Ca^{2+}-sensitizing agent released by certain agonists (Gong *et al.* 1995; Gailly *et al.* 1997), can also activate Rho-kinase (Fu *et al.* 1998; Feng *et al.* 1999) and may contribute an ancillary pathway of RhoA-mediated Ca^{2+} sensitization. *In vitro*, arachidonic acid dissociates the regulatory from the catalytic subunit of MLCP (Gong *et al.* 1992) leading to a

several-fold reduction in myosin-targeted phosphatase activity.

The Ca^{2+}-sensitizing Rho/Rho-kinase pathway can be inhibited at several stages: at the receptor by antagonists to the activating agonist, by hydrolysis of bound GTP to GDP facilitated by GAPs, by complexation of free RhoA with GDI (for smooth muscle, see Longenecker *et al.* 1999), through deactivation/inhibition of Rho-kinase, and by site-specific dephosphorylation of $M_{110\text{-}130}$. RhoA-induced Ca^{2+} sensitization can also be inhibited by Rnd1, a physiological RhoA antagonist that inhibits Ca^{2+} sensitization induced by carbachol, GTPγS, and recombinant RhoA (Loirand *et al.* 1999). Rnd1 is a prenylated Rho-related protein that is constitutively GTP bound and associated with the cell membrane; its expression is stimulated by progesterone or oestrogen. It is, therefore, important to realize that contractions initiated, maintained and terminated through the RhoA/Rho-kinase pathway are the result of a multiple-step process having complex kinetics.

Feedback inhibition of Rho/Rho-kinase-mediated Ca^{2+} sensitization may occur through inhibition of MLCK, because GTPγS and carbachol also increase MLCK phosphorylation of an inhibitory site (Tang *et al.* 1993). This may be due to MLCK's inhibitory site being dephosphorylated by MLCP or by another protein phosphatase that is inhibited by a G-protein-coupled mechanism, or to phosphorylation and inhibition of MLCK by PAK, a serine/threonine kinase activated by other Rho-subfamily GTPases, Cdc42 and Rac (Sanders *et al.* 1999). Val 12 Cdc42 and L61 Rac1 (constitutively active mutants) can inhibit Ca^{2+} sensitization of force induced by constitutively active Val 14 RhoA in permeabilized smooth muscle (M. Gong, P. Read, R. Nakamoto, A. V. Somlyo & A. P. Somlyo, unpublished observation). It remains to be determined whether these effects of Rac and Cdc42 are due to activation of PAK.

The Rho/Rho-kinase pathway plays an important physiological role in intact smooth muscle. The tonic phase of agonist-induced contractions, previously ascribed solely to maintained Ca^{2+} influx, is inhibited by a Rho-kinase inhibitor (Uehata *et al.* 1997; Fu *et al.* 1998), by a cell permeant chimera (DC3B) of C3 that selectively inactivates RhoA (Fujihara *et al.* 1997) and by the somewhat less selective toxin B, without any accompanying decrease in $[Ca^{2+}]_i$ (Lucius *et al.* 1998). These results suggest that the slow (tonic) phase of contractions induced by carbachol in intestinal smooth muscles, unaccompanied by increased $[Ca^{2+}]_i$ (Himpens & Somlyo, 1988), reflects the time course of Ca^{2+} sensitization by RhoA/Rho-kinase-mediated inhibition of myosin phosphatase. Identification of physiological and pathological functions of this pathway is greatly facilitated by use of the highly selective, cell permeant Rho-kinase inhibitor Y-27632 (Uehata *et al.* 1997; Fu *et al.* 1998; Yoshii *et al.* 1999).

Rho-kinase regulates myosin II in both smooth muscle and non-muscle by inhibiting dephosphorylation of myosin RLC, but the activity of MLCP can also be modulated by at least two other mechanisms. Protein kinase C (PKC), activated by phorbol esters or diacylglycerol (Jensen *et al.* 1996; Walker *et al.* 1998 and references therein), and a constitutively active PKC (Ikebe & Brozovich, 1996) can enhance contraction at constant $[Ca^{2+}]_i$ by inhibiting myosin phosphatase directly or by phosphorylating an inhibitor (Somlyo *et al.* 1989; Itoh *et al.* 1993). PKC phosphorylates CPI-17, a potent inhibitor (when phosphorylated) of PP-1C (Eto *et al.* 1995; Kitazawa *et al.* 1999; Senba *et al.* 1999). Rho-kinase and PKC can inhibit MLCP, through convergent mechanisms, in both smooth muscle (Jensen *et al.* 1995) and non-muscle cells (Strassheim *et al.* 1999), with PKC playing a minor and transient (Iizuka *et al.* 1999) role in contractile regulation, its extent possibly depending on the agonist and/or cell type involved. Phorbol ester-induced Ca^{2+} sensitization is not inhibited by Y-27632 (Fu *et al.* 1998). Conversely, cyclic nucleotide (cAMP or cGMP)-activated kinases, possibly through the activity of phosphorylated telokin, accelerate dephosphorylation of RLC (Wu *et al.* 1998 and references therein) leading to muscle relaxation. RLC phosphorylation and contraction can be induced in the absence of Ca^{2+} by phosphatase inhibitors, such as microcystin or calyculin (e.g. Gong *et al.* 1995; Walker *et al.* 1998; Kolodney *et al.* 1999; Weber *et al.* 1999), and are inhibited by a promiscuous kinase inhibitor, staurosporin, but not by Y-27632 (Kureishi *et al.* 1999; Iizuka *et al.* 1999). The Ca^{2+}-independent kinases(s) mediating such phosphorylation remain to be identified (Somlyo, 1999). Direct, *in vitro* phosphorylation of RLC by Rho-kinase (Amano *et al.* 1996) plays no significant role *in vivo*, as in the absence of $[Ca^{2+}]_i$ to activate MLCK even massive stimulation of the Rho/Rho-kinase pathway by GTPγS induces minimal and very slow or no RLC phosphorylation and contraction of smooth muscle (Somlyo *et al.* 1989; Iizuka *et al.* 1999; Swärd *et al.* 2000). G-protein-coupled Ca^{2+} sensitization requires the presence of an active, but not necessarily Ca^{2+}-activated, myosin light chain kinase (Iizuka *et al.* 1999), but the Ca^{2+}-independent kinase (Weber *et al.* 1999; reviewed in Somlyo, 1999*a*) is not Rho-kinase (Kureishi *et al.* 1999; Iizuka *et al.* 1999).

Pathological activity of Rho-kinase in smooth muscle has been implicated in experimental hypertension (Uehata *et al.* 1997) and asthma (Chiba *et al.* 1999). The Rho-kinase inhibitor Y-27632 reduces blood pressure of hypertensive rats, without affecting normal blood pressure (Uehata *et al.* 1997). In (experimental) asthma muscarinic Ca^{2+} sensitization of intrapulmonary bronchial smooth muscle is enhanced and expression of RhoA is increased (Chiba *et al.* 1999). Tumour necrosis factor, implicated in asthma, also increases Ca^{2+} sensitivity of bronchial smooth muscles (Parris *et al.* 1999), but the participation of Rho in this process is not yet known. Thrombin-stimulated vascular smooth muscle migration (Seasholtz *et al.* 1999) and endothelial contraction (Essler *et al.* 1999) are also inhibited by C3 and by Y-27632, suggesting that the Rho/Rho-kinase pathway may play a role in atheromatous plaque formation and post-angioplasty re-stenosis.

The importance of Rho family proteins in cytoskeletal organization of non-muscle cells and the specific role of RhoA·GTP in stimulating stress-fibre formation have been known for some time (Paterson *et al.* 1990; reviewed in Hall, 1994; Lim *et al.* 1996; Narumiya *et al.* 1996), although the connection between these effects and inhibition of myosin phosphatase was recognized only as the result of observations on smooth muscle (reviewed in Somlyo & Somlyo, 1994; Somlyo *et al.* 1999*a*). An ever increasing literature now documents the important role of the Rho/Rho-kinase–myosin phosphatase inhibition pathway in a variety of non-muscle cells, including platelets (Suzuki *et al.* 1999), neuronal (Majumdar *et al.* 1998) and endothelial cells (Vouret-Craviari *et al.* 1998; Essler *et al.* 1998). A highly important pathological consequence of inhibition of myosin phosphatase by Rho-kinase is its enhancement of the motility of malignant cells: their migration, transcellular invasion and metastasis, effects that can be inhibited by the Rho-kinase inhibitor Y-27632 (Itoh *et al.* 1999; Somlyo *et al.* 1999*b*).

We conclude that the RhoA/Rho-kinase pathway plays major physiological and pathophysiological roles through modulation of the activity of smooth and non-muscle myosin II, by inhibiting, as originally suggested (Somlyo *et al.* 1989), the dephosphorylation of myosin II.

Abo, A., Webb, M. R., Grogan, A. & Segal, A. W. (1994). Activation of NADPH oxidase involves the dissociation of $p21^{rac}$ from its inhibitory GDP/GTP exchange protein (rhoGDI) followed by its translocation to the plasma membrane. *Biochemical Journal* **298**, 585–591.

Adelstein, R. S. & Conti, M. A. (1975). Phosphorylation of platelet myosin increases actin-activated myosin ATPase activity. *Nature* **256**, 597–598.

Alessi, D., MacDougall, L. K., Sola, M. M., Ikebe, M. & Cohen, P. (1992). The control of protein phosphatase-1 by targeting subunits: the major myosin phosphatase in avian smooth muscle is a novel form of protein phosphatase-1. *European Journal of Biochemistry* **210**, 1023–1035.

Amano, M., Ito, M., Kimura, K., Fukata, Y., Chihara, K., Nakano, T., Matsuura, Y. & Kaibuchi, K. (1996). Phosphorylation and activation of myosin by Rho-associated kinase (Rho-kinase). *Journal of Biological Chemistry* **271**, 20246–20249.

Andrea, J. E. & Walsh, M. P. (1992). Protein kinase C of smooth muscle. *Hypertension* **20**, 585–595.

Bokoch, G. M., Bohl, B. P. & Chuang, T. H. (1994). Guanine nucleotide exchange regulates membrane translocation of Rac/Rho GTP-binding proteins. *Journal of Biological Chemistry* **269**, 31674–31679.

Bourmeyster, N., Stasia, M.-J., Garin, J., Gagnon, J., Boquet, P. & Vignais, P. V. (1992). Copurification of Rho protein and the Rho-GDP dissociation inhibitor from bovine neutrophil cytosol. Effect of phosphoinositides on rho ADP-ribosylation by the C3 exoenzyme of *Clostridium botulinum*. *Biochemistry* **31**, 12863–12869.

Bradley, A. B. & Morgan, K. G. (1987). Alterations in cytoplasmic calcium sensitivity during porcine coronary artery contractions as detected by aequorin. *Journal of Physiology* **385**, 437–448.

Cherfils, J. & Chardin, P. (1999). GEFs: structural basis for their activation of small GTP-binding proteins. *Trends in Biological Sciences* **24**, 306–311.

Chiba, Y., Takada, Y., Miyamoto, S., Mitsui-Saito, M., Karaki, H. & Misawa, M. (1999). Augmented acetylcholine-induced, Rho-mediated Ca^{2+}-sensitization of bronchial smooth muscle contraction in antigen-induced airway hypertensive rats. *British Journal of Pharmacology* **127**, 597–600.

Croxton, T. L., Lande, B. & Hirshman, C. A. (1998). Role of G proteins in agonist-induced Ca^{2+} sensitization of tracheal smooth muscle. *American Journal of Physiology* **275**, L748–755.

Essler, M., Amano, M., Kruse, H.-J., Kaibuchi, K., Weber, P. C. & Aepfelbacher, M. (1998). Thrombin inactivates myosin light chain phosphatase via Rho and its target Rho kinase in human endothelial cells. *Journal of Biological Chemistry* **273**, 21867–21874.

Essler, M., Retzer, M., Bauer, M., Heemskerk, J. W., Aepfelbacher, M. & Siess, W. (1999). Mildly oxidized low density lipoprotein induces contraction of human endothelial cells through activation of Rho/Rho kinase and inhibition of myosin light chain phosphatase. *Journal of Biological Chemistry* **274**, 30361–30363.

Eto, M., Ohmori, T., Suzuki, M., Furuya, K. & Morita, F. (1995). A novel protein phosphatase-1 inhibitory protein potentiated by protein kinase C. Isolation from porcine aorta media and characterization. *Journal of Biochemistry* **118**, 1104–1107.

Feng, J., Ito, M., Ichikawa, K., Isaka, N., Nishikawa, M., Hartshorne, D. J. & Nakano, T. (2000). Inhibitory phosphorylation site for Rho-associated kinase on the smooth muscle myosin phosphatase target. *Journal of Biological Chemistry* (in the Press).

Feng, J., Ito, M., Kureishi, Y., Ichikawa, K., Amano, M., Isaka, N., Okawa, K., Iwamatsu, A., Kaibuchi, K., Hartshorne, D. J. & Nakano, T. (1999). Rho-associated kinase of chicken gizzard smooth muscle. *Journal of Biological Chemistry* **274**, 3744–3752.

Fu, X., Gong, M., C., Jia, T., Somlyo, A. V. & Somlyo, A. P. (1998). The effects of the Rho-kinase inhibitor Y-27632 on arachidonic acid-, GTPγS-, and phorbol ester-induced Ca^{2+}-sensitization of smooth muscle. *FEBS Letters* **440**, 183–187.

Fujihara, H., Walker, L. A., Gong, M. C., Lemichez, E., Boquet, P., Somlyo, A. V. & Somlyo, A. P. (1997). Inhibition of RhoA translocation and calcium sensitization by *in vivo* ADP-ribosylation with the chimeric toxin DC3B. *Molecular Biology of the Cell* **8**, 2437–2447.

Gailly, P., Gong, M. C., Somlyo, A. V. & Somlyo, A. P. (1997). Possible role of atypical protein kinase C activated by arachidonic acid in Ca^{2+} sensitization of rabbit smooth muscle. *Journal of Physiology* **500**, 95–110.

Gailly, P., Wu, X., Haystead, T. A. J., Somlyo, A. P., Cohen, P. T. W., Cohen, P. & Somlyo, A. V. (1996). Regions of the 100-kDa regulatory subunit M_{110} required for regulation of myosin-light-chain-phosphatase activity in smooth muscle. *European Journal of Biochemistry* **239**, 326–332.

Gallagher, P. J., Herring, B. P. & Stull, J. T. (1997). Myosin light chain kinases. *Journal of Muscle Research and Cell Motility* **18**, 1–16.

Gohla, A., Offermanns, S., Wilkie, T. M. & Schultz, G. (1999). Differential involvement of $G\alpha_{12}$ and $G\alpha_{13}$ in receptor-mediated stress fiber formation. *Journal of Biological Chemistry* **274**, 17901–17907.

Gong, M. C., Fuglsang, A., Alessi, D., Kobayashi, S., Cohen, P., Somlyo, A. V. & Somlyo, A. P. (1992). Arachidonic acid inhibits myosin light chain phosphatase and sensitizes smooth muscle to calcium. *Journal of Biological Chemistry* **267**, 21492–21498.

Gong, M. C., Fujihara, H., Somlyo, A. V. & Somlyo, A. P. (1997). Translocation of rhoA associated with Ca^{2+}-sensitization of smooth muscle. *Journal of Biological Chemistry* **272**, 10704–10709.

Gong, M. C., Iizuka, K., Nixon, G., Browne, J. P., Hall, A., Eccleston, J. F., Sugai, M., Kobayashi, S., Somlyo, A. V. & Somlyo, A. P. (1996). Role of guanine nucleotide-binding proteins – ras-family or trimeric or both – in Ca^{2+} sensitization of smooth muscle. *Proceedings of the National Academy of Sciences of the USA* **93**, 1340–1345.

Gong, M. C., Kinter, M. T., Somlyo, A. V. & Somlyo, A. P. (1995). Arachidonic acid and diacylglycerol release associated with inhibition of myosin light chain dephosphorylation in rabbit smooth muscle. *Journal of Physiology* **486**, 113–122.

Gosser, Y. Q., Nomanbhoy, T. K., Aghazadeh, B., Manor, D., Combs, C., Cerione, R. A. & Rosen, M. K. (1997) C-terminal domain of Rho GDP-dissociation inhibitor directs N-terminal inhibitory peptide to GTPases. *Nature* **387**, 814–819.

Hall, A. (1994). Small GTP-binding proteins and the regulation of the actin cytoskeleton. *Annual Review of Cell Biology* **10**, 31–54.

Hart, M. J., Jiang, X., Kozasa, T., Roscoe, W., Singer, W. D., Gilman, A. G., Sternweis, P. C. & Bollag, G. (1998). Direct stimulation of the guanine nucleotide exchange activity of p115 RhoGEF by $G\alpha_{13}$. *Science* **280**, 2112–2114.

Hartshorne, D. J. (1987). Biochemistry of the contractile proteins in smooth muscle. In *Physiology of the Gastrointestinal Tract*, 2nd edn, ed. Johnson, L.R., pp. 423–482. Raven Press, New York

Hartshorne, D. J., Ito, M. & Erdödi, F. (1998). Myosin light chain phosphatase: subunit composition, interactions and regulation. *Journal of Muscle Research and Cell Motility* **19**, 325–341.

Haystead, C. M. M., Gailly, P., Somlyo, A. P., Somlyo, A. V. & Haystead, T. A. J. (1995). Molecular cloning and functional expression of a recombinant 72·5 kDa fragment of the 110 kDa regulatory subunit of smooth muscle protein phosphatase 1M. *FEBS Letters* **377**, 123–127.

Himpens, B., Kitazawa, T. & Somlyo, A. P. (1990). Agonist dependent modulation of the Ca^{2+} sensitivity in rabbit pulmonary artery smooth muscle. *Pflügers Archiv* **417**, 21–28.

Himpens, B. & Somlyo, A. P. (1988). Free-calcium and force transients during depolarization and pharmacomechanical coupling in guinea-pig smooth muscle. *Journal of Physiology* **395**, 507–530.

Hirshman, C. A. & Emala, C. W. (1999). Actin reorganization in airway smooth muscle cells involves G_q and $G_{i\text{-}2}$ activation of Rho. *American Journal of Physiology* **277**, L653–661.

Ichikawa, K., Ito., M. & Hartshorne, D. J. (1996). Phosphorylation of the large subunit of myosin phosphatase and inhibition of phosphatase activity. *Journal of Biological Chemistry* **271**, 4733–4740.

Iizuka, K., Yoshii, A., Samizo, K., Tsukagoshi, H., Ishizuka, T., Dobashi, K., Nakazawa, T. & Mori, M. (1999). A major role for the Rho-associated coiled coil forming protein kinase in G-protein-mediated Ca^{2+}-sensitization through inhibition of myosin phosphatase in rabbit trachea. *British Journal of Pharmacology* **128**, 925–933.

Ikebe, M. & Brozovich, F. V. (1996). Protein kinase C increases force and slows relaxation in smooth muscle: evidence for regulation of the myosin light chain phosphatase. *Biochemical and Biophysical Research Communications* **225**, 370–376.

Ishizaki, T., Maekawa, M., Fujisawa, K., Okawa, K., Iwamatsu, A., Fujita, A., Watanabe, N., Saito, Y., Kakizuka, A., Morii, N. & Narumiya, S. (1996). The small GTP-binding protein Rho binds to and activates a 160 kDa Ser/Thr protein kinase homologous to myotonic dystrophy kinase. *EMBO Journal* **15**, 1885–1893.

Itagaki, M., Komori, S., Unno, T., Syuto, B. & Ohashi, H. (1995). Possible involvement of a small G-protein sensitive to exoenzyme C3 of *Clostridium botulinum* in the regulation of myofilament Ca^{2+} sensitivity in β-escin skinned smooth muscle of guinea pig ileum. *Japanese Journal of Pharmacology* **67**, 1–7.

Itoh, H., Shimomura, A., Okubo, S., Ichikawa, K., Ito, M., Konishi, T. & Nakano, T. (1993). Inhibition of myosin light chain phosphatase during Ca^{2+}-independent vasocontraction. *American Journal of Physiology* **265**, C1319–1324.

Itoh, K., Yoshioka, K., Akedo, H., Uehata, M., Ishizaki, T. & Narumiya, S. (1999). An essential part for Rho-associated kinase in the transcellular invasion of tumor cells. *Nature Medicine* **5**, 221–225.

Jensen, P. E., Gong, M. C., Somlyo, A. V. & Somlyo, A. P. (1996). Separate upstream and convergent downstream pathways of G-protein and phorbol ester-mediated Ca^{2+}-sensitization of myosin light chain phosphorylation in smooth muscle. *Biochemical Journal* **318**, 469–475.

Katoh, H., Aoki, J., Yamaguchi, Y., Kitano, Y., Ichikawa, A. & Negishi, M. (1998). Constitutively active $G\alpha_{12}$, $G\alpha_{13}$, and $G\alpha_q$ induce Rho-dependent neurite retraction through different signaling pathways. *Journal of Biological Chemistry* **273**, 28700–28707.

Kimura, K., Ito, M., Amano, M., Chihara, K., Fukata, Y., Nakafuku, M., Yamamori, B., Feng, J., Nakano, T., Okawa, K., Iwamatsu, A. & Kaibuchi, K. (1996). Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). *Science* **273**, 245–248.

Kitazawa, T., Masuo, M. & Somlyo, A. P. (1991). G-protein-mediated inhibition of myosin light-chain phosphatase in vascular smooth muscle. *Proceedings of the National Academy of Sciences of the USA* **88**, 9307–9310.

Kitazawa, T., Takizawa, N., Ikebe, M. & Eto, M. (1999). Reconstitution of protein kinase C-induced contractile Ca^{2+}-sensitization in Triton X-100 demembranated rabbit arterial smooth muscle. *Journal of Physiology* **520**, 139–152.

Klages, B., Brandt, U., Simon, M. I., Schultz, G. & Offermanns, S. (1999). Activation of G_{12}/ G_{13} results in shape change and Rho/Rho-kinase-mediated myosin light chain phosphorylation in mouse platelets. *Journal of Cellular Biology* **14**, 745–754.

Kobayashi, S., Gong, M. C., Somlyo, A. V. & Somlyo, A. P. (1991). Ca^{2+} channel blockers distinguish between G protein-coupled pharmacomechanical coupling Ca^{2+}-release and Ca^{2+}-sensitization. *American Journal of Physiology* **260**, C364–370.

Kolodney, M. S., Thimgan, M. S., Honda, H. M., Tsai, G. & Yee, H. F. Jr (1999). Ca^{2+}-independent myosin II phosphorylation and contraction in chicken embryo fibroblasts. *Journal of Physiology* **515**, 87–92.

Kozasa, T., Jiang, X., Hart, M. J., Sternweis, P. M., Singer, W. D., Gilman, A. G., Bollag, G. & Sternweis, P. C. (1998). p115 RhoGEF, a GTPase activating protein for $G\alpha_{12}$ and $G\alpha_{13}$. *Science* **280**, 2109–2111.

Kureishi, Y., Ita, M., Feng, J., Okinaka, T., Isaka, N. & Nakano, T. (1999). Regulation of Ca^{2+}-independent smooth muscle contraction by alternative staurosporine-sensitive kinase. *European Journal of Pharmacology* **376**, 315–320.

Leung, T., Manser, E., Tan, L. & Lim, L. (1995). A novel serine/threonine kinase binding the Ras-related RhoA GTPase which translocates the kinase to peripheral membranes. *Journal of Biological Chemistry* **270**, 29051–29054.

Lim, L., Manser, E., Leung, T. & Hall, C. (1996) Regulation of phosphorylation pathways by p21 GTPases. The p21 Ras-related Rho subfamily and its role in phosphorylation signalling pathways. *European Journal of Biochemistry* **242**, 171–185.

Loirand, G., Cario-Toumaniantz, C., Chardin, P. & Pacaud, P. (1999). The Rho-related protein Rnd1 inhibits Ca^{2+} sensitization of rat smooth muscle. *Journal of Physiology* **516**, 825–834.

Longenecker, K., Read, P, Derewenda, U., Dauter, Z., Liu, X., Garrard, S., Walker, L., Somlyo, A. V., Nakamoto, R. K., Somlyo, A. P. & Derewenda, Z. S. (1999). How RhoGDI binds Rho. *Acta Crystallographica* **D55**, 1503–1515.

Lucius, C., Arner, A., Steusloff, A., Troschka, M., Hofmann, F., Aktories, K. & Pfitzer, G. (1998). *Clostridium difficile* toxin B inhibits carbachol-induced force and myosin light chain phosphorylation in guinea-pig smooth muscle: role of Rho proteins. *Journal of Physiology* **506**, 83–93.

Majumdar, M., Seasholtz, T. M., Goldstein, D., de Lanerolle, P. & Brown, J. H. (1998). Requirement for Rho-mediated myosin light chain phosphorylation in thrombin-stimulated cell rounding and its dissociation from mitogenesis. *Journal of Biological Chemistry* **273**, 10099–10106.

Matsui, T., Amano, M., Yamamoto, T., Chihara, K., Nakafuku, M., Ito, M., Nakano, T., Okawa, K., Iwamatsu, A. & Kaibuchi, K. (1996). Rho-associated kinase, a novel serine/threonine kinase, as a putative target for the small GTP binding protein Rho. *EMBO Journal* **15**, 2208–2216.

Murányi, A., Erdödi, F., Ito, M., Gergely, P. & Hartshorne, D. J. (1998). Identification and localization of myosin phosphatase in human platelets. *Biochemical Journal* **330**, 225–231.

Nakai, K., Suzuki, Y., Khira, H., Wada, H., Fujioka, M., Ito, M., Nakano, T., Kaibuchi, K., Shiku, H. & Nishikawa, M. (1997). Regulation of myosin phosphatase through phosphorylation of the myosin-binding subunit in platelet activation. *Blood* **90**, 3936–3942.

Narumiya, S. (1996). The small GTPase Rho: cellular functions and signal transduction. *Journal of Biochemistry* **120**, 215–228.

Noda, M., Yasuda-Fukazawa, C., Moriishi, K., Kato, T., Okuda, T., Kurokawa, K. & Takuwa, Y. (1995) Involvement of rho in GTPγS-induced enhancement of phosphorylation of 20 kDa myosin light chain in vascular smooth muscle cells: inhibition of phosphatase activity. *FEBS Letters* **367**, 246–250.

Otto, B., Steusloff, A., Just, I., Aktories, K., Pfitzer, G. (1996). Role of Rho proteins in carbachol-induced contractions in intact and permeabilized guinea-pig intestinal smooth muscle. *Journal of Physiology* **496**, 317–329.

Parris, J. R. M., Cobban, H. J., Littlejohn, A. F., Macewan, D. J. & Nixon, G. F. (1999). Tumour necrosis factor-α activates a calcium sensitization pathway in guinea-pig bronchial smooth muscle. *Journal of Physiology* **518**, 561–569.

Paterson, H. F., Self, A. J., Garrett, M. D., Just, I., Aktories, K. & Hall, A. (1990). Microinjection of recombinant p21rho induces rapid changes in cell morphology. *Journal of Cellular Biology* **111**, 1001–1007.

Read, P. W., Liu, X., Longenecker, K., DiPierro, C. G., Walker, L. A., Somlyo, A. V., Somlyo, A. P. & Nakamoto, R. K. (2000). Human RhoA/RhoGDI complex expressed in yeast: GTP exchange is sufficient for translocation of RhoA to liposomes. *Protein Science* (in the Press).

Sanders, L. C., Matsumura, F., Bokoch, G. M. & de Lanerolle, P. (1999). Inhibition of myosin light chain kinase by p21-activated kinase. *Science* **283**, 2083–2085.

Seasholtz, T. M., Majumdar, M., Kaplan, D. D. & Brown, J. H. (1999). Rho and Rho kinase mediate thrombin-stimulated vascular smooth muscle cell DNA synthesis and migration. *Circulation Research* **84**, 1186–1193.

Sehr, P., Joseph, G., Genth, H., Just, I., Pick, E. & Aktories, K. (1998). Glucosylation and ADP ribosylation of Rho proteins: effects on nucleotide binding, GTPase activity, and effector coupling. *Biochemistry* **37**, 5296–5304.

Senba, S., Eto, M. & Yazawa, M. (1999). Identification of trimeric myosin phosphatase (PP1M) as a target for a novel PKC-potentiated protein phosphatase-1 inhibitory protein (CPI17) in porcine aorta smooth muscle. *Journal of Biochemistry* **125**, 354–362.

Shimizu, H., Ito, M., Miyahara, M., Ichikawa, K., Okubo, S., Konishi, T., Naka, M., Tanaka, T., Hirano, K., Hartshorne, D. J. & Nakano, T. (1994). Characterization of the myosin-binding subunit of smooth muscle myosin phosphatase. *Journal of Biological Chemistry* **269**, 30407–304011.

Shirazi, A., Iizuka, K., Fadden, P., Mosse, C., Somlyo, A. P., Somlyo, A. V. & Haystead, T. A. J. (1994). Purification and characterization of the mammalian myosin light chain phosphatase holoenzyme: The differential effects of the holoenzyme and its subunits on smooth muscle. *Journal of Biological Chemistry* **269**, 31598–31606.

Somlyo, A. P. (1999). Kinases, myosin phosphatase and Rho proteins: curiouser and curiouser. *Journal of Physiology* **516**, 630.

Somlyo, A. P., Kitazawa, T., Himpens, B., Matthijs, G., Horiuti, K., Kobayashi, S., Goldman, Y. E. & Somlyo, A. V. (1989). Modulation of Ca^{2+}-sensitivity and of the time course of contraction in smooth muscle: a major role of protein phosphatases? *Advances in Protein Phosphatases* **5**, 181–195.

Somlyo, A. P. & Somlyo, A. V. (1994). Signal transduction and regulation in smooth muscle. *Nature* **372**, 231–236.

Somlyo, A. P., Wu, X., Walker, L. A. & Somlyo, A. V. (1999*a*) Pharmacomechanical coupling: the role of calcium, G-proteins, kinases and phosphatases. *Review of Physiological and Biochemical Pharmacology* **134**, 201–234.

Somlyo, A. V., Bradshaw, D., Ramos, S., Murphy, C., Myers, C. E. & Somlyo, A. P. (1999*b*). Rho-kinase inhibitor Y-27632 alters human prostate cancer cell morphology, inhibits migration and reduces myosin phosphorylation. *Molecular Biology of the Cell* **10** (suppl.), 416a.

Somlyo, A. V. & Somlyo, A. P. (1968). Electromechanical and pharmacomechanical coupling in vascular smooth muscle. *Journal of Pharmacology and Experimental Therapeutics* **159**, 129–145.

Strassheim, D., May, L. G., Varker, K. A., Puhl, H. L., Phelps, S. H., Porter, R. A., Aronstam, R. S., Noti, J. D. & Williams, C. L. (1999). M_3 muscarinic acetylcholine receptors regulate cytoplasmic myosin by a process involving RhoA and requiring conventional protein kinase C isoforms. *Journal of Biological Chemistry* **274**, 18675–18685.

Suzuki, Y., Yamamoto, M., Wada, H., Ito, M., Nakano, T., Sasaki, Y., Narumiya, S., Skiku, H. & Nishikawa, M. (1999). Agonist-induced regulation of myosin phosphatase activity in human platelets through activation of Rho-kinase. *Blood* **93**, 3408–3417.

Swärd, K., Dreja, K., Susnjar, M., Hellstrand, P., Hartshorne, D. J. & Walsh, M. P. (2000). Inhibition of Rho-associated kinase blocks agonist-induced Ca^{2+} sensitization of myosin phosphorylation and force in guinea pig ileum. *Journal of Physiology* **522**, 33–49.

Taggart, M. J., Lee, Y. H. & Morgan, K. G. (1999). Cellular redistribution of RKCα, rhoA, and ROKα following smooth muscle agonist stimulation. *Experimental Cell Research* **251**, 92–101.

Tan, J. L., Ravid, S. & Spudich, J. A. (1992). Control of nonmuscle myosins by phosphorylation. *Annual Review of Biochemistry* **61**, 721–759.

Tang, D. C., Kubota, Y., Kamm, K. E. & Stull, J. T. (1993). GTP gamma S-induced phosphorylation of myosin light chain kinase in smooth muscle. *FEBS Letters* **331**, 272–275.

Trinkle-Mulcahy, L., Ichikawa, K., Hartshorne, D. J., Siegman, M. J. & Butler, T. M. (1995). Thiophosphorylation of the 130-kDa subunit is associated with a decreased activity of myosin light chain phosphatase in alpha-toxin-permeabilized smooth muscle. *Journal of Biological Chemistry* **270**, 18191–18194.

Uehata, M., Ishizuki, T., Satoh, H., Ono, T., Kawahara, T., Morishita, T., Tamakawa, H., Yamagami, K., Inui, J., Maekawa, M. & Narumiya, S. (1997). Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. *Nature* **389**, 990–994.

Vouret-Craviari, V., Boquet, P., Pouysségur, J. & Van Obberghen-Schilling, E. (1998). Regulation of the actin cytoskeleton by thrombin in human endothelial cells: role of Rho proteins in endothelial barrier function. *Molecular Biology of the Cell* **9**, 2639–2653.

Walker, L. A., Gailly, P., Jensen, P. E., Somlyo, A. V. & Somlyo, A. P. (1998). The unimportance of being (protein kinase C) epsilon. *FASEB Journal* **12**, 813–821.

Weber, L. P., Van Lierop, J. E. & Walsh, M. P. (1999). Ca^{2+}-independent phosphorylation of myosin in rat caudal artery and chicken gizzard myofilaments. *Journal of Physiology* **516**, 805–824.

Wei, Y., Zhang, Y., Derewenda, U., Liu, X., Minor, W., Nakamoto, R. K., Somlyo, A. V., Somlyo, A. P. & Derewenda, Z. (1997). Crystal structure of RhoA-GDP and its functional implications. *Nature Structural Biology* **4**, 699–703.

Wu, X., Haystead, T. A. J., Nakamoto, R. K., Somlyo, A. V. & Somlyo, A. P. (1998). Acceleration of myosin light chain dephosphorylation and relaxation of smooth muscle by telokin: synergism with cyclic nucleotide-activated kinase. *Journal of Biological Chemistry* **273**, 11362–11369.

Yoshii, A., Iizuka, K., Dobashi, K., Horie, T., Harada, T., Nakazawa, T. & Mori, M. (1999). Relaxation of contracted rabbit tracheal and human bronchial smooth muscle by Y-27632 through inhibition of Ca^{2+}-sensitization. *American Journal of Respiratory Cell and Molecular Biology* **20**, 1190–1200.

Zhou, Y., Hirano, K., Sakihara, C., Nishimura, J. & Kanaide, H. (1999). NH_2-terminal fragments of the 130 kDa subunit of myosin phosphatase increase the Ca^{2+} sensitivity of porcine renal artery. *Journal of Physiology* **516**, 55–65.

Acknowledgements

The authors' research covered in this review was supported by NIH grants HL19242 and HL48807. We thank Dr K. Longenecker for Fig. 2, Dr D. Hartshorne for constructive comments, and Ms B. Nordin for preparation of the manuscript.

Corresponding author

A. P. Somlyo: Department of Molecular Physiology and Biological Physics, University of Virginia Health System, PO Box 800736, Charlottesville, VA 22908-0736, USA.

Email: aps2n@virginia.edu